L Number	Hits	Search Text	DB	Time stamp
1	17215	tuberculosis or mycobacter$ or bovis	USPAT; US-PGPUB	2004/05/18 12:36
2	295	allel$ near5 exchang$	USPAT; US-PGPUB	2004/05/18 12:36
3	106	(tuberculosis or mycobacter$ or bovis) and (allel$ near5 exchang$)	USPAT; US-PGPUB	2004/05/18 12:36
4	1000367	@pd>20011123	USPAT; US-PGPUB	2004/05/18 12:37
5	77	((tuberculosis or mycobacter$ or bovis) and (allel$ near5 exchang$)) and @pd>20011123	USPAT; US-PGPUB	2004/05/18 12:37
6	738355	@rlad<19990708	USPAT; US-PGPUB	2004/05/18 12:37
8	0	(allel$ near5 exchang$) and leprea	USPAT; US-PGPUB	2004/05/18 12:38
7	33	(((tuberculosis or mycobacter$ or bovis) and (allel$ near5 exchang$)) and @pd>20011123) and @rlad<19990708	USPAT; US-PGPUB	2004/05/18 12:38

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31805 MYCOBACTER?/BI
21140 MYCOBACTER?/AB
29469 TUBERCULOSIS/BI
19697 TUBERCULOSIS/AB
4851 BOVIS/BI
4005 BOVIS/AB
1946 LEPRAE/BI
1505 LEPRAE/AB
L1 46278 (MYCOBACTER? OR TUBERCULOSIS OR BOVIS OR LEPRAE)/BI,AB

=> s (allel?(5a)exchang?)/bi,ab
83252 ALLEL?/BI
75730 ALLEL?/AB
623027 EXCHANG?/BI
517124 EXCHANG?/AB
L2 456 (ALLEL?(5A)EXCHANG?)/BI,AB

=> s l1 and l2
L3 51 L1 AND L2

=> s l3 not 2004/py
404538 2004/PY
L4 48 L3 NOT 2004/PY

=> s l4 not 2003/py
1138604 2003/PY
L5 36 L4 NOT 2003/PY

=> s l5 not 2002/py
1127576 2002/PY
L6 28 L5 NOT 2002/PY

=> s l6 not 2001/py
1076693 2001/PY
L7 19 L6 NOT 2001/PY

=> s l7 not 2000/py
1015294 2000/PY
L8 16 L7 NOT 2000/PY

=> d his

(FILE 'HOME' ENTERED AT 12:44:29 ON 18 MAY 2004)

FILE 'CAPLUS' ENTERED AT 12:44:40 ON 18 MAY 2004
L1 46278 S (MYCOBACTER? OR TUBERCULOSIS OR BOVIS OR LEPRAE)/BI,AB
L2 456 S (ALLEL?(5A)EXCHANG?)/BI,AB
L3 51 S L1 AND L2
L4 48 S L3 NOT 2004/PY
L5 36 S L4 NOT 2003/PY
L6 28 S L5 NOT 2002/PY
L7 19 S L6 NOT 2001/PY
L8 16 S L7 NOT 2000/PY

=> d l8 1-16 bib ab

L8 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:532831 CAPLUS Full-text
DN 131:282163
TI Comparison of the construction of unmarked deletion mutations in
Mycobacterium smegmatis, **Mycobacterium bovis** Bacillus Calmette-Guerin, and **Mycobacterium tuberculosis** H37Rv by **allelic exchange**
AU Pavelka, Martin S., Jr.; Jacobs, William R., Jr.
CS Department of Microbiology and Immunology, Albert Einstein College of
Medicine, Bronx, NY, 10461, USA
SO Journal of Bacteriology (1999), 181(16), 4780-4789
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB Until recently, genetic anal. of **Mycobacterium tuberculosis**, the causative agent of **tuberculosis**, was hindered by a lack of methods for gene disruptions and **allelic exchange.** Several groups have described different methods for disrupting genes marked with antibiotic resistance determinants in the slow-growing organisms **Mycobacterium bovis** bacillus Calmette-Guerin (BCG) and M. **tuberculosis.** In this study, we described the first report of using a **mycobacterial** suicidal plasmid bearing the counterselectable marker sacB for the **allelic exchange** of unmarked deletion mutations in the chromosomes of two substrains of M. **bovis** BCG and M. **tuberculosis** H37Rv. In addition, our comparison of the recombination frequencies in these two slow-growing species and that of the fast-growing organism **Mycobacterium** smegmatis suggests that the homologous recombination machinery of the three species is equally efficient. The mutants constructed here have deletions in the lysA gene, encoding meso-diaminopimelate decarboxylase, an enzyme catalyzing the last step in lysine biosynthesis. We observed striking differences in the lysine auxotrophic phenotypes of these three species of **mycobacteria.** The M. smegmatis mutant can grow on lysine-supplemented defined medium or complex rich medium, while the BCG mutants grow only on lysine-supplemented defined medium and are unable to form colonies on complex rich medium. The M. **tuberculosis** lysine auxotroph requires 25-fold more lysine on defined medium than do the other mutants and is dependent upon the detergent Tween 80. The mutants described in this work are potential vaccine candidates and can also be used for studies of cell wall biosynthesis and amino acid metabolism
RE.CNT 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:180266 CAPLUS Full-text
DN 131:83474
TI The genetics of **Mycobacterium tuberculosis**
AU Vijaya, S.
CS Department of Microbiology and Cell Biology, Indian Institute of Science,
Bangalore, 560 012, India
SO Journal of Genetics (1998), 77(2 & 3), 123-128
CODEN: JOGNAU; ISSN: 0022-1333
PB Indian Academy of Sciences
DT Journal; General Review
LA English
AB A review with 23 refs. that discusses the current status of conditionally replicating plasmid and transposon vectors, and their application in generating targeted mutations in **mycobacteria.** Gene manipulation in **Mycobacterium tuberculosis** has been slow in coming of age owing to the inherent difficulties associated with working on this aerosol-transmitted pathogen, in addition to the paucity of mol. tools such as plasmids and transposons. One of the early approaches to overcome these difficulties was the development of phasmids, which combined the properties of phages and plasmids and allowed introduction of recombinant genes into **mycobacteria.** The lone plasmid pAL5000 of **mycobacteria** has been exploited to its fullest potential in the construction of a plethora of vectors. Above all, the single most important achievement has been the development of elegant and innovative approaches to overcome the problem of illegitimate recombination which threatened the success of **allelic-exchange** mutagenesis in the slow-growing pathogenic **mycobacterial** species.
RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:159255 CAPLUS Full-text
DN 130:308953
TI RecA-mediated gene conversion and aminoglycoside resistance in strains
heterozygous for rRNA
AU Prammananan, Therdsak; Sander, Peter; Springer, Burkhard; Bottger, Erik C.
CS Institut fur Medizinische Mikrobiologie, Medizinische Hochschule Hannover,
Hannover, 30623, Germany
SO Antimicrobial Agents and Chemotherapy (1999), 43(3), 447-453
CODEN: AMACCQ; ISSN: 0066-4804
PB American Society for Microbiology
DT Journal
LA English
AB Clin. resistance to aminoglycosides in general is due to enzymic drug modification. Mutational alterations of the small ribosomal subunit rRNA have recently been found to mediate acquired resistance in bacterial pathogens in vivo. In this study, the authors investigated the effect of 16S rRNA heterozygosity (wild-type [wt] and mutant [mut] operons at position 1408 [1408wt/1408mut]) on aminoglycoside resistance. Using an integrative vector, they introduced a single copy of a mutated rRNA operon (1408 A→G) into **Mycobacterium** smegmatis, which carries two chromosomal wild-type rRNA operons; the resultant transformants exhibited an aminoglycoside-sensitive phenotype. In contrast, introduction of the mutated rRNA operon into an M. smegmatis rrnB knockout strain carrying a single functional chromosomal wild-type rRNA operon resulted in aminoglycoside-resistant transformants. Subsequent anal. by DNA sequencing and RNase protection assays unexpectedly demonstrated a homozygous mutant genotype, rRNAmut/rRNAmut, in the

resistant transformants. To investigate whether RecA-mediated gene conversion was responsible for the aminoglycoside-resistant phenotype in the rRNAwt/rRNAmut strains, recA mutant strains were generated by **allelic exchange** techniques. Transformation of the recA rrnB M.smegmatis mutant strains with an integrative vector expressing a mutated rRNA operon (Escherichia coli position 1408 A→G) resulted in transformants with an aminoglycoside-sensitive phenotype. Subsequent anal. showed stable heterozygosity at 16S rRNA position 1408 with a single wild-type allele and a single resistant allele. These results demonstrate that rRNA-mediated mutational resistance to aminoglycosides is recessive.

RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:109758 CAPLUS Full-text
DN 130:264739
TI RecA protein of **Mycobacterium tuberculosis** possesses pH-dependent homologous DNA pairing and strand **exchange** activities: implications for **allele exchange** in **mycobacteria**
AU Vaze, Moreshwar B.; Muniyappa, K.
CS Department of Biochemistry, Indian Institute of Science, Bangalore, 560 012, India
SO Biochemistry (1999), 38(10), 3175-3186
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB To gain insights into inefficient **allele exchange** in **mycobacteria**, we compared homologous pairing and strand exchange reactions promoted by RecA protein of **Mycobacterium tuberculosis** to those of Escherichia coli RecA protein. The extent of single-stranded binding protein (SSB)-stimulated formation of joint mols. by MtRecA was similar to that of EcRecA over a wide range of pH values. In contrast, strand exchange promoted by MtRecA was inhibited around neutral pH due to the formation of DNA networks. At higher pH, MtRecA was able to overcome this constraint and, consequently, displayed optimal strand exchange activity. Order of addition expts. suggested that SSB, when added after MtRecA, was vital for strand exchange. Significantly, with shorter duplex DNA, MtRecA promoted efficient strand exchange without network formation in a pH-independent fashion. Increase in the length of duplex DNA led to incomplete strand exchange with concomitant rise in the formation of intermediates and networks in a pH-dependent manner. Treatment of purified networks with S1 nuclease liberated linear duplex DNA and products, consistent with a model in which the networks are formed by the invasion of hybrid DNA by the displaced linear single-stranded DNA. Titration of strand exchange reactions with ATP or salt distinguished a condition under which the formation of networks was blocked, but strand exchange was not significantly affected. We discuss how these results relate to inefficient **allele exchange** in **mycobacteria.**

RE.CNT 81 THERE ARE 81 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:620025 CAPLUS Full-text
DN 129:326873
TI Investigation of **mycobacterial** recA function: protein introns in the RecA of pathogenic **mycobacteria** do not affect competency for homologous recombination
AU Frischkorn, Klaus; Sander, Peter; Scholz, Matthias; Teschner, Kerstin; Prammananan, Therdsak; Bottger, Erik C.
CS Institut fur Medizinische Mikrobiologie, Medizinische Hochschule Hannover, Hannover, 30623, Germany
SO Molecular Microbiology (1998), 29(5), 1203-1214
CODEN: MOMIEE; ISSN: 0950-382X
PB Blackwell Science Ltd.
DT Journal
LA English
AB The recA locus of pathogenic **mycobacteria** differs from that of non-pathogenic species in that it contains large intervening sequences termed protein introns or inteins that are excised by an unusual protein-splicing reaction. In addition, a high degree of illegitimate recombination has been observed in the pathogenic **Mycobacterium tuberculosis** complex. Homologous recombination is the main mechanism of integration of exogenous nucleic acids in M. smegmatis, a non-pathogenic **mycobacterium** species that carries an inteinless RecA and is amenable to genetic manipulations. To investigate the function of recA in **mycobacteria**, recA- strains of M. smegmatis were generated by **allelic exchange** techniques. These strains are characterized (i) by increased sensitivity towards DNA-damaging agents [ethylmethylsulfonate (EMS), mitomycin C, UV irradiation] and (ii) by the inability to integrate nucleic acids by homologous recombination. Transformation efficiencies using integrative or replicative vectors were not affected in recA- mutants, indicating that in **mycobacteria** RecA does not affect plasmid uptake or replication. Complementation of the recA- mutants with the recA from M. **tuberculosis** restored resistance towards EMS, mitomycin C and UV irradiation Transformation of the complemented strains with suicide vectors targeting the pyrF gene resulted in numerous **allelic exchange** mutants. From these data, we conclude that the intein apparently does not interfere with RecA function, i.e. with respect to competency for homologous recombination, the RecAs from pathogenic and non-pathogenic **mycobacteria** are indistinguishable.

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:534633 CAPLUS Full-text
DN 129:243403
TI The 16-kDa α-crystallin (Acr) protein of **Mycobacterium tuberculosis** is required for growth in macrophages
AU Yuan, Ying; Crane, Deborah D.; Simpson, R. Mark; Zhu, Ya Qi; Hickey, Mark J.; Sherman, David R.; Barry, Clifton E., III
CS Tuberculosis Research Unit, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, 59840, USA
SO Proceedings of the National Academy of Sciences of the United States of America (1998), 95(16), 9578-9583
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB Although the 16-kDa α-crystallin homolog of M. **tuberculosis** (MTB) is the dominant protein produced by

stationary phase cultures in vitro, it is undetectable in logarithmically growing cultures. By growing bacilli at defined oxygen concns., acr transcription was shown to be strongly induced by mildly hypoxic conditions. Acr expression also was induced during the course of in vitro infection of macrophages. The acr gene was replaced with a hygromycin resistance cassette by **allelic exchange** in MTB H37Rv. The resulting Δacr :: hpt strain was shown to be equivalent to wild-type H37Rv in in vitro growth rate and infectivity but was impaired for growth in both mouse bone marrow derived macrophages and THP-1 cells. In addition to its proposed role in maintenance of long-term viability during latent, asymptomatic infections, these results establish a role for the Acr protein in replication during initial MTB infection.

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:237172 CAPLUS Full-text
DN 129:24128
TI An acyl-CoA synthase (acoas) gene adjacent to the mycocerosic acid
synthase (mas) locus is necessary for mycocerosyl lipid synthesis in
Mycobacterium tuberculosis var. **bovis** BCG
AU Fitzmaurice, Ann M.; Kolattukudy, Pappachan E.
CS Neurobiotechnol. Cent. Deps. Biochem. Medicinal Biochem., Ohio State
Univ., Columbus, OH, 43210, USA
SO Journal of Biological Chemistry (1998), 273(14), 8033-8039
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB An open reading frame, ORF3, first identified adjacent to the mycocerosic acid synthase gene in **Mycobacterium bovis** BCG encodes a protein with acyl-CoA synthase (ACoAS) activity. Genes homologous to acoas are found adjacent to other multifunctional polyketide synthase genes in the **mycobacterial** genome. To test whether these gene products are necessary to esterify the fatty acids generated by the adjacent polyketide synthase gene products, the acoas gene was disrupted in M. **Bovis** BCG using a suicide vector containing the acoas gene with an internal deletion and the hygromycin-resistant gene as selection marker. **Allelic exchange** at the acoas locus was confirmed by Southern hybridization and polymerase chain reaction amplification of both flanking regions expected from homologous recombination. Immunoblot anal. indicated that the 65-kDa AcoAS protein product was absent in the mutant. Chromatog. anal. of lipids derived from [1-14C]propionate showed that the mutant did not produce mycocerosyl lipids, although it produced normal levels of mycocerosic acid synthase. These results suggest that ACoAS is involved in the synthesis of mycocerosyl lipids of the **mycobacterial** cell wall.

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:634467 CAPLUS Full-text
DN 127:315253
TI Efficient **allelic exchange** and transposon mutagenesis in **Mycobacterium tuberculosis**
AU Pelicic, Vladimir; Jackson, Mary; Reyrat, Jean-Marc; Jacobs, William R.,
Jr.; Gicquel, Brigitte; Guilhot, Christophe
CS Unite Genetique Mycobacterienne, Institut Pasteur, Paris, F-75724, Fr.
SO Proceedings of the National Academy of Sciences of the United States of
America (1997), 94(20), 10955-10960
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB A better understanding of **Mycobacterium tuberculosis** virulence mechanisms is highly dependent on the design of efficient mutagenesis systems. A system enabling the pos. selection of insertional mutants having lost the delivery vector was developed. It uses ts-sacB vectors, which combine the counterselective properties of the sacB gene and a **mycobacterial** thermosensitive origin of replication and can therefore be efficiently counterselected on sucrose at 39°C. This methodol. allowed the construction of M. **tuberculosis** transposition mutant libraries. Greater than 106 mutants were obtained, far exceeding the number theor. required to obtain at least one insertion in every nonessential gene. This system is also efficient for gene exchange mutagenesis as demonstrated with the purC gene: 100% of the selected clones were **allelic exchange** mutants. Therefore, a single, simple methodol. has enabled us to develop powerful mutagenesis systems, the lack of which was a major obstacle to the genetic characterization of M. **tuberculosis.**

L8 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:482757 CAPLUS Full-text
DN 127:233226
TI Attenuation and vaccine potential of aroQ mutants of Corynebacterium
pseudotuberculosis
AU Simmons, Cameron P.; Hodgson, Adrian L. M.; Strugnell, Richard A.
CS CRC for Vaccine Technology and Department of Microbiology and Immunology,
University of Melbourne, Parkville, 3052, Australia
SO Infection and Immunity (1997), 65(8), 3048-3056
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Corynebacterium pseudotuberculosis, a gram-pos. intracellular bacterial pathogen, is the etiol. agent of the disease caseous lymphadenitis (CLA) in both sheep and goats. Attenuated mutants of C. pseudotuberculosis have the potential to act as novel live veterinary vaccine vectors. The authors have cloned and sequenced the aroB and aroQ genes from C. pseudotuberculosis C231. By **allelic exchange**, aroQ mutants of both C231, designated CS100, and a pld mutant strain TB521, designated CS200, were constructed. Infection of BALB/c mice indicated that introduction of the aroQ mutation into C231 and TB521 attenuated both strains. In sublethally infected BALB/c mice, both CS100 and CS200 were cleared from spleens and livers by day 8 postinfection. The in vivo persistence of these strains was increased when the intact aroQ gene was supplied on a plasmid in trans. Mice infected with TB521 harbored bacteria in organs at least till day 8 postinfection without ill effect. When used as a vaccine, only the maximum tolerated dose of CS100 had the capacity to protect mice from homologous challenge. Vaccination with TB521 also elicited protective immunity, and this was associated with gamma interferon (IFN-γ) production from splenocytes stimulated 7 days postvaccination. The role of IFN-γ in controlling primary

infections with C. pseudotuberculosis was examined in mice deficient for the IFN-γ receptor (IFN-γR-/- mice). IFN-γR-/- mice cleared an infection with CS100 but were significantly more susceptible than control littermates to infection with C231 or TB521. These studies support an important role for IFN-γ in control of primary C. pseudotuberculosis infections and indicate that aroQ mutants remain attenuated even in immunocompromised animals. This is the first report of an aroQ mutant of a bacterial pathogen, and the results may have implications for the construction of aromatic mutants of **Mycobacterium tuberculosis** for use as vaccines.

L8 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:757085 CAPLUS Full-text
DN 126:43236
TI Introducing mutations into a chromosomal rRNA gene using a genetically
modified eubacterial host with a single rRNA operon
AU Sander, Peter; Prammananan, Therdsak; Boettger, Erik C.
CS Inst. Med. Mikrobiol., Medizinische Hochschule Hannover, Hannover, 30625,
Germany
SO Molecular Microbiology (1996), 22(5), 841-848
CODEN: MOMIEE; ISSN: 0950-382X
PB Blackwell
DT Journal
LA English
AB Gene-inactivation techniques were employed to construct a eubacterial organism harboring a single functional rRNA operon. This mutant of **Mycobacterium** smegmatis permits replacement of the single remaining rRNA operon with a homologous fragment from a vector-borne gene. By homologous recombination with the chromosome a plasmid-borne rDNA segment with resistance markers substitutes for the corresponding region of the chromosomal rRNA operon, resulting in a homogeneous population of mutated ribosomes in the cell. As a first result the authors demonstrate that the single allelic knock-out strain allows for isolation of rRNA mutants with a drug-resistant phenotype, circumventing the problem of recessivity which prohibits the isolation of such mutants in organisms with multiple rRNA operons. Subsequently, by **allelic exchange** expts., it was demonstrated that the rRNA mutation found indeed confers drug resistance in vivo. This system provides intriguing potential for the study of the structure and function of rRNAs.

L8 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:684483 CAPLUS Full-text
DN 125:319086
TI Positive selection of **allelic exchange** mutants in **Mycobacterium bovis** BCG
AU Pelicic, Vladimir; Reyrat, Jean-Marc; Gicquel, Brigitte
CS Unite de Genetique Mycobacterienne, Institut Pasteur, Paris, F-75015, Fr.
SO FEMS Microbiology Letters (1996), 144(2-3), 161-166
CODEN: FMLED7; ISSN: 0378-1097
PB Elsevier
DT Journal
LA English
AB SacB expression is lethal to **mycobacteria** in the presence by sucrose. It can therefore serve as a counter-selectable marker for pos. selection of gene replacement events as demonstrated in the fast-growing **Mycobacterium** smegmatis. With this methodol., a sucrose counter-selectable vector was used to deliver, into the **Mycobacterium bovis** BCG genome, an inactivated copy (ureC::KM) of the ureC gene encoding the **mycobacterial** urease. A two-step selection procedure on 2% sucrose allowed the pos. selection of gene exchange mutants. This technique should thus be extremely useful for the genetic anal. of pathogenic **mycobacteria.**

L8 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:536279 CAPLUS Full-text
DN 125:216810
TI Urease activity does not contribute dramatically to persistence of **Mycobacterium bovis** Bacillus Calmette-Guerin
AU Reyrat, Jean-Marc; Lopez-Ramirez, Gloria; Ofredo, Catherine; Gicquel,
Brigitte; Winter, Nathalie
CS Unite Genetique Mycobacterienne, Inst. Pasteur, Paris, Fr.
SO Infection and Immunity (1996), 64(9), 3934-3936
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Multiplication of BCGure-, an isogenic urease-neg. mutant of M. **bovis** BCG constructed by **allelic exchange** was examined in human macrophages and mice. Although ureolytic activity was not essential to BCGure- growth, a slight decrease in the multiplication and persistence of the mutated strain compared with wild-type BCG was observed in lungs of infected mice.

L8 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:392684 CAPLUS Full-text
DN 125:77694
TI Generation of unmarked directed mutations in **mycobacteria,** using
sucrose counter-selectable suicide vectors
AU Pelicic, Vladimir; Reyrat, Jean-Marc; Gicquel, Brigitte
CS Unite Genet. Mycobacterienne, CNRS URA, Paris, F-75015, Fr.
SO Molecular Microbiology (1996), 20(5), 919-925
CODEN: MOMIEE; ISSN: 0950-382X
PB Blackwell
DT Journal
LA English
AB The expression of sacB, the Bacillus subtilis gene encoding levansucrase, is lethal to **mycobacteria** in the presence of 10% sucrose. In this study, we describe the use of sacB as a marker for pos. selection of gene-replacement events into **Mycobacterium** smegmatis. A sucrose counter-selectable suicide plasmid was used to deliver an inactivated copy of the pyrF gene (pyrF::Km) into the M. smegmatis genome. Only uracil auxotroph clones, resulting from replacement of the endogenous pyrF allele, survived in a one-step selection on plates containing kanamycin and 10% sucrose. This demonstrated that selection on sucrose against the maintenance of the vector bearing the sacB gene is 100% efficient, enabling the pos. selection of **allelic-exchange** mutants. Two-step selection is also feasible; it was used to construct unmarked pyrF mutants in which the gene was inactivated by a frameshift mutation. This method of generating unmarked, directed mutations is rapid and simple, making it a powerful tool for the genetic characterization of **mycobacteria.**

L8 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:19783 CAPLUS Full-text
DN 124:108870
TI **Allelic exchange** in **Mycobacterium tuberculosis** with long linear recombination substrates
AU Balasubramanian, V.; Pavelka, Martin S., Jr.; Bardarov, Stoyan

S.; Martin,
Jean; Weisbrod, Torin R.; McAdam, Ruth A.; Bloom, Barry R.; Jacobs,
William R., Jr.
CS Howard Hughes Med. Inst., Albert Einstein Coll. Med., Bronx, NY, 10461,
USA
SO Journal of Bacteriology (1996), 178(1), 273-9
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB Genetic studies of **Mycobacterium tuberculosis** have been greatly hampered by the inability to introduce specific chromosomal mutations. Whereas the ability to perform **allelic exchanges** has provided a useful method of gene disruption in other organisms, in the clin. important species of **mycobacteria**, such as M. **tuberculosis** and **Mycobacterium bovis**, similar approaches have thus far been unsuccessful. In this communication, we report the development of a shuttle mutagenesis strategy that involves the use of long linear recombination substrates to reproducibly obtain recombinants by **allelic exchange** in M. **tuberculosis.** Long linear recombination substrates, approx. 40 to 50 kb in length, were generated by constructing libraries in the excisable cosmid vector pYUB328. The cosmid vector could be readily excised from the recombinant cosmids by digestion with PacI, a restriction endonuclease for which there exist few, if any, sites in **mycobacterial** genomes. A cosmid containing the **mycobacterial** leuD gene was isolated, and a selectable marker conferring resistance to kanamycin was inserted into the leuD gene in the recombinant cosmid by interplasmic recombination in Escherichia coli. A long linear recombination substrate containing the insertionally mutated leuD gene was generated by PacI digestion. Electroporation of this recombination substrate containing the insertionally mutated leuD allele resulted in the genration of leucine auxotrophic mutants by homologous recombination in 6% of the kanamycin-resistant transformants for both the Erdman and H37Rv strains of M. **tuberculosis.** The ability to perform **allelic exchanges** provides an important approach for investigating the biol. of this pathogen as well as developing new live-cell M. **tuberculosis**-based vaccines.

L8 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1995:805914 CAPLUS Full-text
DN 124:2012
TI The urease locus of **Mycobacterium tuberculosis** and its utilization for the demonstration of **allelic exchange** in **Mycobacterium bovis** bacillus Calmette-Guerin
AU Reyrat, Jean-Marc; Berthet, Francois-Xavier; Gicquel, Brigitte
CS Cent. Natl. Recherche Sci., Inst. Pasteur 25, Paris, F-75724, Fr.
SO Proceedings of the National Academy of Sciences of the United States of
America (1995), 92(19), 8768-72
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB The ureABC genes of **Mycobacterium tuberculosis** were cloned. By using a set of degenerate primers corresponding to a conserved region of the urease enzyme (EC 3.5.1.5), a fragment of the expected size was amplified by PCR and was used to screen a M. **tuberculosis** cosmid library. Three open reading frames with extensive similarity to the urease genes from other organisms were found. The locus was mapped on the chromosome, using an ordered M. **tuberculosis** cosmid library. A suicide vector containing a ureC gene disrupted by a kanamycin marker (aph) was used to construct a urease-neg. **Mycobacterium bovis** bacillus Calmette-Guerin mutant by **allelic exchange** involving replacement of the ureC with the aph::ureC construct. To our knowledge, **allelic exchange** has not been reported previously in the slow-growing **mycobacteria.** Homologous recombination will be an invaluable genetic tool for deciphering the mechanisms of **tuberculosis** pathogenesis, a disease that causes 3 + 106 deaths a year worldwide.

L8 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1993:17437 CAPLUS Full-text
DN 118:17437
TI Temperature-sensitive mutants of the **Mycobacterium** plasmid pAL5000
AU Guilhot, Christophe; Gicquel, Brigitte; Martin, Carlos
CS Unite Genie Microbiol., Inst. Pasteur, Paris, F-75015, Fr.
SO FEMS Microbiology Letters (1992), 98(1-3), 181-6
CODEN: FMLED7; ISSN: 0378-1097
DT Journal
LA English
AB Two plasmids were isolated as thermosensitive replicons following in vitro mutagenesis of pB4, a pAL5000 derivative **mycobacteria**/Escherichia coli shuttle plasmid. Plasmids pCG59 and pCG63 replicate at 30° but not at 39°. This will allow their utilization for transposon delivery, site-specific integration, or **allele exchange** .

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